AD_____

Award Number: DAMD17-02-1-0473

TITLE: Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk

PRINCIPAL INVESTIGATOR: Doctor Lynn C. Hartmann

CONTRACTING ORGANIZATION: Mayo Clinic Rochester
Rochester, Minnesota 55905

REPORT DATE: June 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | June 2004 | Annual (1 Jun 03-31 May 04) |

**4. TITLE AND SUBTITLE**
Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk

**5. FUNDING NUMBERS**
DAMD17-02-1-0473

**6. AUTHOR(S)**
Doctor Lynn C. Hartmann

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Mayo Clinic Rochester
Rochester, Minnesota 55905

E-Mail: hartmann.lynn@mayo.edu

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**
Original contains color plates. All DTIC reproductions will be in black and white.

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT (Maximum 200 Words)**

Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. Unfortunately only a minority of the ~ 200,000 women who are diagnosed with breast cancer in the US each year are recognized as being at significantly increased risk. The purpose of this Center is to bring molecular risk prediction for breast cancer into the clinical arena. This will require progress on three fronts of scientific endeavor: (i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk. We have made good progress on our tissue repository and have begun our biomarker studies. We completed the follow-up of the 1982-91 group (n = 5,181) and are in process with the 1967-81 group (n = 6,102). A total of 762 cases of breast cancer were identified in this 25 year cohort. We established a relational database in which data are entered on an ongoing basis. Benign histopathology has been characterized for 80% of the 25 year cohort. We have begun the process of collecting fresh tissue for culturing in vitro.

**14. SUBJECT TERMS**
Benign breast disease; biomarkers

**15. NUMBER OF PAGES**
32

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

**Table of Contents**

## INTRODUCTION

This is our second year Center of Excellence report; however, it details a total of only 16 months of work involving human subjects because of delays in start-up of funding (detailed in timeline on page 13). The purpose of our Center of Excellence is to bring molecular risk prediction for breast cancer into the clinical arena. There are three main areas of scientific activity within this proposed Center: 1) the establishment of a large tissue repository from a retrospective cohort of women with benign breast disease (BBD) (1967-91) with complete and long-term clinical follow-up to identify those who developed breast cancer (cases) and those who did not (controls); 2) the application of potential biomarkers of risk to this archival tissue set; and, 3) the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD. The Center includes a multi-institutional team of basic scientists, pathologists, epidemiologists, clinicians, statisticians, and advocates (Mayo Clinic; UCSF; Wayne State).

## BODY

### Task 1: Establish Retrospective Cohort of BBD and Nested Case-Control Study

Complete follow-up of 1982-91 group:

Last year we reported the process by which we used the following Mayo Clinic databases to identify our study cohort: Surgical Index, Pathology Index, Medical Index and Tumor Registry. At the end of the screening process, 5,340 women were considered eligible for the study, 259 of whom were cases.

Surveys were mailed to these 5,340 women or their next-of-kin if the woman was known to be dead; 4354 (82%) of the surveys were returned. Six percent (n=319) of the women were lost to follow-up, 7% refused participation, and 5% could not be reached on telephone follow-up to complete the survey. The survey captured factors associated with an increased risk of breast cancer as well as information concerning additional breast biopsies and the development of breast cancer.

The number of women who were eligible during this period of time was further refined based on questionnaire responses (e.g. diagnosis of breast cancer outside of Mayo Clinic within 6 months of her breast biopsy, prophylactic mastectomies prior to the breast biopsy) and pathologist reading of the slides. Currently, 5,181 women are eligible during this time period. Based on identification of breast cancer on the questionnaire and a subsequent review of all the aforementioned Mayo indexes, we have identified 39 additional cases. Thus, we have identified a total of 298 cases in the 1982-91 cohort.

Update follow-up of 1967-81 group

This cohort of women was originally established through an R01 grant to Dr. David Ballard (epidemiology) at Mayo Clinic in the late 1980s. This cohort included 6,943 women with tissue available on 6,805 (98.1%). These women were last followed for subsequent occurrence of breast cancer through 1985; only 138 refused (2.0%) and 339 (5.0%) were lost to follow-up. A total of 294 cases of breast cancer were identified through 1985. The number of women who were eligible during

this period was further refined based on pathologist reading of the slides and current study criteria. Currently, 6,102 women are eligible during this time period. Based on a recent review of the Mayo Indexes (Medical Index and Tumor Registry) used to identify the 1982-91 group, we have identified 170 additional cases of breast cancer in this 1967-81 group, resulting in a total of 464 cases. Thus, we have a total of 464 + 298 = 762 cases of breast cancer to date in the entire 25 year cohort.

Surveys were recently mailed to the 6,102 women in the 1967-81 group. We are currently working on second mailings and locating women who no longer live at the address that we have on file.

Validate reported breast cancers

Charts were reviewed to validate all breast cancer diagnoses. For women in both study cohorts (i.e., across the entire 25-year period) who were diagnosed with breast cancer somewhere other than Mayo Clinic (n=271, 36%), a contact was initiated to obtain permission to access medical records associated with their breast cancer diagnosis and their breast cancer tissue. To date, we have received tissue blocks on 40% (n=109) of the 271 women diagnosed elsewhere. We have a slide only on an additional 18% (n = 49). We did not request permission on five (2%) of these women as they or their next-of-kin did not complete the questionnaire. No tissue was available for six (2%) of these women and permission was denied by 7% (n=19) women or their next-of-kin. The remaining 83 (31%) are in process.

Match breast cancer controls to cases

The following matching procedure was performed independently for the two study periods, 1967 to 1981 and 1982 to 1991. Any eligible woman that developed breast cancer at least 6 months after breast biopsy was considered a case. Eligible women who did not develop breast cancer were considered controls.

One case was matched to 10 controls by age at breast biopsy and year of biopsy using the greedy matching algorithm developed by Rosenbaum. Ten controls were identified for each case to allow for the inclusion of 2 matches with available tissue. The closest matches will be used. The algorithm begins by randomly sorting the cases and controls. The distance between the first case and each potential control is determined using a weighted sum of the absolute difference between their age at biopsy and the year of their biopsy. All pairings where the follow-up time of the potential control is less than the time from breast biopsy to breast cancer diagnosis of the case is eliminated from consideration. The control closest to the case (i.e. the one with the smallest distance) is chosen as the case's first control. The algorithm then moves on to the second case in the listing and computes the distance between that case and all remaining potential controls. This process continues until all cases have their first control. The second control is then found and the process is repeated a total of 10 times to obtain 10 matches for each case. The result of this allocation scheme is that the case-control matches on the first pass are the closest (in terms of age at biopsy and year of biopsy). With each successive pass, the distance between the case and additional control grows.

Construct test set for preliminary evaluation of biomarkers

A subset of 124 cases and their two closest controls was chosen from the entire study period, 1967 to

1991, to serve as a test set. This set will be used to ascertain a point and interval estimate of the prevalence of a candidate marker among the cases, as well as the risk of breast cancer among those with the candidate marker relative to those without the marker. The most promising candidate markers will be assessed further in the validation data set.

The first step in constructing the test set was to determine the proportion of cases that occurred in each calendar year (1967-1991). This proportion was used to determine the composition of the number of cases to be included in the test set for a particular year. Once the number to be selected from a particular calendar year was determined, that number was randomly selected from among the cases in that calendar year.

Construct validation set from remaining breast cancer cases

The remaining cases and their controls will serve as a validation set.

**Task 2: Biomarkers In Archived Tissues from Cases and Controls**

Retrieve tissue blocks of BBD specimens for cases and controls

To date we have retrieved benign tissue blocks from the Mayo Clinic archives for 612 of the 762 cases (80%). For the test set, we have tissue blocks on two matched controls for each case. We currently have 27% of the matched control blocks for the validation set (this process is ongoing). Archived tissue of paraffin blocks and slides for these patients was obtained from the Mayo Clinic Tissue Registry. For tracking purposes, the pathology numbers, assigned at biopsy to the blocks and corresponding slides, are entered on SAS screens in the data set for each patient. A tracking system in the database identifies the location of those blocks and slides at any point in time (see description of database in Task 4 below).

Characterize benign histopathology

Our study pathologists, Drs. Carol Reynolds and Dan Visscher, have to date characterized the benign histology for 8,957 of the potential 11,283 specimens. The general categories and proportions are: non-proliferative disease without atypia (67%); proliferative disease without atypia (28%); and proliferative disease with atypia (3%). The remaining 2% are unresolved, that is another slide will need to be cut for reading. Additionally, the pathologists are reviewing the slides for the following detail: apocrine metaplasia, ductal hyperplasia, lobular hyperplasia, calcifications, cysts, duct ectasia, fibroadenoma, fibrosis, intra-ductal papilloma, radial scars, sclerosing adenosis, columnar alteration, mucocele like tumors and atrophy.

Prepare slides for biomarker analyses

Slide preparation is occuring in the Tissue Acquisition Core of the Mayo Clinic Cancer Center (MCCC). As blocks need to be sectioned for slides, an identifying code number is etched on the slides. A software system, using bar codes and bar code readers, to track the location of these slides and blocks has been purchased and installed. (This process is described below in the relational database section.)

Many of the older tissues are embedded in paraffin blocks of odd size and/or the paraffin is too brittle to cut. This has required us to re-embed these older specimens. To date we have re-embedded 1,179 blocks in new paraffin, and continue to do so as blocks are identified.

Establishment of laboratory procedures

We tested a variety of procedures with a "technical set" of tissue from women not eligible for our study to verify the appropriateness of the process. Specifically, we cut and stained benign breast tissue from 47 non-study women. We put the slides in long-term cold room storage to ascertain that labels would continue to adhere and the selected antibody dilution continued to work under those conditions. We then stained slides from the 47 cases in the technical set that had been cold-stored for 3 to 6 months. The staining quality was assessed as "excellent" by Dr. Visscher.

Additionally, we tested the Sybase Database program, adding various components to optimize the tracking of each sample as it became available. The bar coded label contains a coded patient identification number and information about the block that was used for slide preparation.

Cutting and staining of test set samples

Preparatory sample work included the screening of samples by cutting current H&Es as well as re-embedding about 460 blocks. The DOD Sybase Database program was optimized and finalized to assist with the tracking of each sample as it became available. Block boxes were labeled with a 1-D bar code block box label that tracks which blocks are present in the box. Fifteen slides were printed with a 2-D bar code encoding block number, slide number, immunostain, section thickness, and slide box location. To date about 350 blocks have been cut and stored in this set. About 15 blocks that did not meet the criteria have been pulled from the test set after cutting, leaving about 335 blocks in the finalized test set. The number 1 and number 15 slides have been stained for H&E and sent to Dr. Visscher, the pathologist. Slide number 2 has been set aside for γ-tubulin immunofluorescence labeling. This labeling will be initiated after all the H&E slides have been marked by the pathologist. These slides are stored in the cold room to be handled as optimized by use of the technical set in 2003. The number 3 slides have been labeled with Cyclin D1 (Biocare Medical, catalogue #CP236B) as per the technical set optimization. The COX-2 (DAKO Cytomation, Carpentaria, CA) labeling was done at a dilution of 1:100 on the number 4 slides. The number 5 slides were labeled with a double cocktail for Ki-67/Caspase-3 (Biocare Medical, catalogue # PPM240DSAA). The staining for a given antibody was done within a twenty-four hour time frame for all 335 slides using two DAKO autostainers after pooling newly-purchased reagents with like lot numbers together, such as the primary antibody at the optimized dilution, the detection system reagent and the chromogen reagent, keeping the conditions constant for all 335 slides per antibody. To date, we have H&E stained 700 slides and completed IHC staining on 1005 slides in the test set. The slides are stored in groups according to immunostain (i.e., all of the number 3 slides together, the number 4 slides, and so on) to match the stain in bar coded slide boxes. The remaining slides number 6 through number 14 slides are kept in the cold room until further stains have been selected. To date we have about 3000 slides stored for future use. All 335 blocks sectioned have had sufficient tissue present in all 15 slides.

IHC Protocols

We replaced the use of the Cayman COX-2 antibody with a new COX-2 mouse monoclonal from DAKO Cytomation (Carpentaria, CA). After HIER by use of EDTA, the labeling was done using the primary at 1:100 dilution and visualized by use of the DAKO Cytomation EnVision+, HRP, AEC+ system. The addition of the Ki-67(M) +Caspase-3(R) Double stain cocktail from Biocare Medical was implemented. This is purchased as a predilute. HIER is done by use of EDTA. Visualization is completed by use of the Biocare MACH2 HRP-ALP Double secondary reagent. The Ki-67 is seen by the Biocare's Cardassian DAB and the Caspase-3 by use of the Biocare Vulcan Fast Red Chromogen kit. We continue to label for Cyclin D1 as we did with the technical set using the Biocare primary antibody.

Digital Image Capture

All stained slides are being digitized using the BLISS Virtual Microscopy system (Bacus Laboratories, Chicago, IL). Prior to digitization, the pathologist circles areas of interest on slide number 1 (Figure 1). Corresponding areas are then marked on the IHC-stained slides from the same block. Each slide is scanned in its entirety at low magnification, and the areas of interest are scanned at higher magnification (Figure 1). These image files are linked to the Sybase database program. To date, slides from seven of the 335 blocks have been digitized. Now that the system has been refined, we anticipate digitizing slide sets at a rate of 15 blocks per week (=75 slides per week). Using this system, the entire image files can be accessed electronically from any computer within the Mayo system by personnel who have the proper computer file sharing permissions. Once accessed, the user can navigate around the slides, change magnification, and capture screen shots for publication or other purposes. Figure 1 was assembled using this system.

**Task 3: Discovery - *In Vitro* Culturing and Gene Profiling Studies**

Task 3 of our Center grant is directed toward the discovery of new, potentially meaningful biomarkers of risk.

Obtain fresh BBD tissue from patients

This work will be based in prospectively ascertained samples of benign breast disease from Mayo Clinic (Caucasian women) and Wayne State (African American women).

This part of our work has just begun in the last few months at Mayo Clinic. Thus far, we have collected five fresh samples from Mayo Clinic. All five of these samples have grown successfully at UCSF. One factor that has slowed progress on this aim is that many breast biopsies today are performed as core needle biopsies, rather than an open, excisional biopsy. Originally, Dr. Tlsty's team at UCSF needed as much breast tissue as possible to establish cultures, more than was available from a needle biopsy. However, since the original proposal, the UCSF team has refined their techniques and they are now able to grow specimens from a smaller amount of tissue. Thus, we are now pursuing the possibility of using core biopsy material.

For Wayne State the delays were confounded by several factors: (1) there was considerable turnover

among the human subjects specialists at the DOD who were responsible for our grant, (2) there was a bit of a standoff between the DOD and Wayne State on consent form language around institutional coverage for any "research-related risks". Dr. Beitens from the Department of Defense helped us clear these hurdles in early 2004 and a DOD-acceptable consent form was just approved by Wayne State in May. (IRB minutes attached as Appendix A). See Appendix B for a copy of the consent form, brochure and questionnaire used for the collection of fresh tissue.

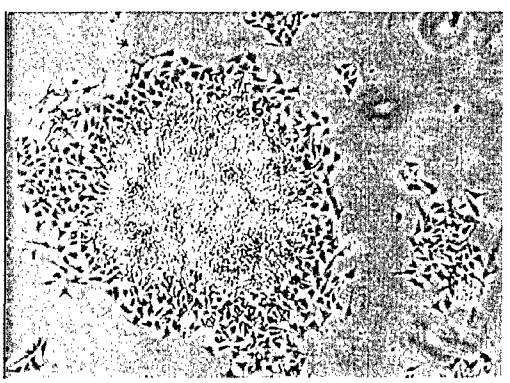
Culture 80 BBD specimens and document growth characteristics

The five samples sent to UCSF from Mayo were sent via FED EX on ice. Once they arrived at UCSF, they were immediately placed in culture medium to dissociate the tissue into organoids and single cells. From the dissociated organoids, UCSF has been able to grow viable mammary epithelial and mammary fibroblast cells. They have been successful with growing all samples received to date. Figure 2 below shows the outgrowth of epithelial and fibroblast cells from the organoid. These cells are subsequently separated via differential trypsinization and propagated as pure cultures. Analysis has shown that these purified populations are not contaminated with other cell types. UCSF is now poised to analyze the proliferation kinetics and epigenetic modifications in the BBD samples to compare with their extensive experience in analyzing reduction mammoplasty tissue. These experiments are in process at the present time.

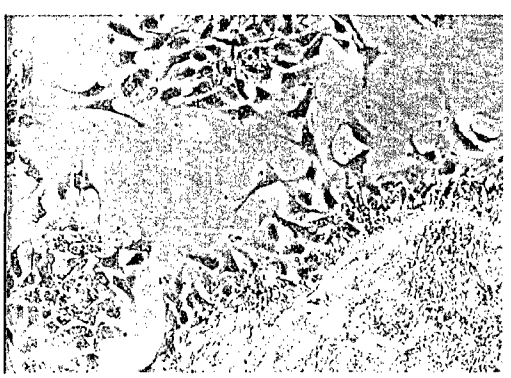
Current analysis of a special subpopulation of variant HMEC from reduction mammoplasty tissue suggests that these cells have attained properties that are similar to those of premalignant breast lesions. UCSF will determine if these characteristics are shared or exacerbated in tissue samples from BBD.

**Figure 2.** Outgrowth of epithelial and fibroblast cells from the organoid

- BBD 0304
- 18 hrs in culture in MEGM media
- 4x magnification



- BBD 0304
- 18 hrs in culture in MEGM media
- 10x magnification



Compare genomic expression levels of DCIS markers in BBD tissues

We have identified 10 cell cycle and cytoskeletal genes (TTK, Cen2, EB1, CD27, GST1, TACC3, SEI1, BCCIP, skb1 homologue, and PCR1) that are over-expressed in DCIS relative to normal breast to use in quantitative real-time PCR studies of benign tissues that will be collected prospectively. Laser capture microdissection will be used to collect benign epithelial cells from which RNA will be isolated. We are awaiting the prospectively obtained tissue samples to proceed with this task. We also have collected 10 fresh frozen DCIS tissues from which we will isolate RNA for gene expression studies. In these studies, we will use Affymetrix gene chips to compare gene expression in DCIS relative to 5 normal breast tissues in order to identify additional genes for real-time PCR of benign breast lesions.

**Task 4: Statistical Analyses**

Establish relational database

Last year we reported on the creation of our Sybase database. We continue to refine this database as we activate various components and the investigators further clarify the data they want to report.

Data entry

We continue to enter data for the various aspects of our study. The following highlights those activities:

- All data from the 4,354 returned questionnaires for the 1982-91 cohort have been entered.
- Next-of-kin information is updated on an ongoing basis. To date, this information has been verified and updated for 426 study participants.
- All the histology data read to date on the 8,957 women's slides have been entered.
- Slides are being entered into the database as we receive them from tissue registry. To date, slides for 10,274 women have been entered.
- Available tissue blocks are being entered into the database as we receive them. To date, available tissue blocks have been entered on 612 cases and 3,022 controls.
- We have documented to the extent possible all 762 breast cancers. We verified the breast cancer by medical records and recorded the histopathology, TNM, date of diagnosis, recurrence information when available, ER, PR, and type of surgery.

We are in the process of doing data clean-up on these sections in preparation for writing our first manuscript.

**KEY RESEARCH ACCOMPLISHMENTS**

We are in the second year of our award. We have begun our biomarker analyses on our test set and have begun to culture fresh tissue in vitro. We have demonstrated that we can ship fresh breast tissue across the country and have it cultured successfully at UCSF. We are beginning to analyze the data for a publication describing the project, especially the histology and descriptive data on

variables such as family history, age at benign breast biopsy and time between breast biopsy and cancer.

## REPORTABLE OUTCOMES

- Abstract, AACR, 2003 by Hartmann et al. on the 1982-91 cohort (see Appendix C).
- Abstract, AACR, 2004 by Hartmann et al. (see Appendix D).

## CONCLUSIONS

Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. Unfortunately only a minority of the ~ 200,000 women who are diagnosed with breast cancer in the US each year are recognized as being at significantly increased risk. The purpose of this Center is to bring molecular risk prediction for breast cancer into the clinical arena. This will require progress on three fronts of scientific endeavor: (i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk.

This month, June 2004, marks only the 16$^{th}$ month since our approval for human subjects work went through the DOD (see timeline, below). Nevertheless, we have demonstrated solid progress on all three aims. Specifically the follow-up of our 1982-91 group is complete. We have made good progress on our tissue repository and have begun our biomarker studies. Our pathologist has completed the characterization of the benign histopathology for 80% of our 25-year cohort. We have begun the collection of fresh tissue for culturing in vitro at UCSF.

## REFERENCES

AACR abstract
AACR abstract #2

## TIMELINE

| | |
|---|---|
| 6/01 | Grant submitted |
| 12/01 | Notice of funding |
| 5/02 | Funding released for non-human subjects portion of the grant<br>• Began work on the establishment of the retrospective cohort, identification of breast cancer cases<br>• Retrieved slides and began characterization of benign histopathy |
| 2/3/03 | DOD approval for all Mayo Clinic portions of the grant; issues remain with Wayne State consent form. Work began on portions involving human subjects at Mayo Clinic. |
| 6/03 | Report of year 1 (includes only 4 months of work involving human subjects); continued issues with Wayne State consent form |
| 6/04 | Report of year 2 (includes only 16 months of work involving human subjects); new contact at Wayne State |

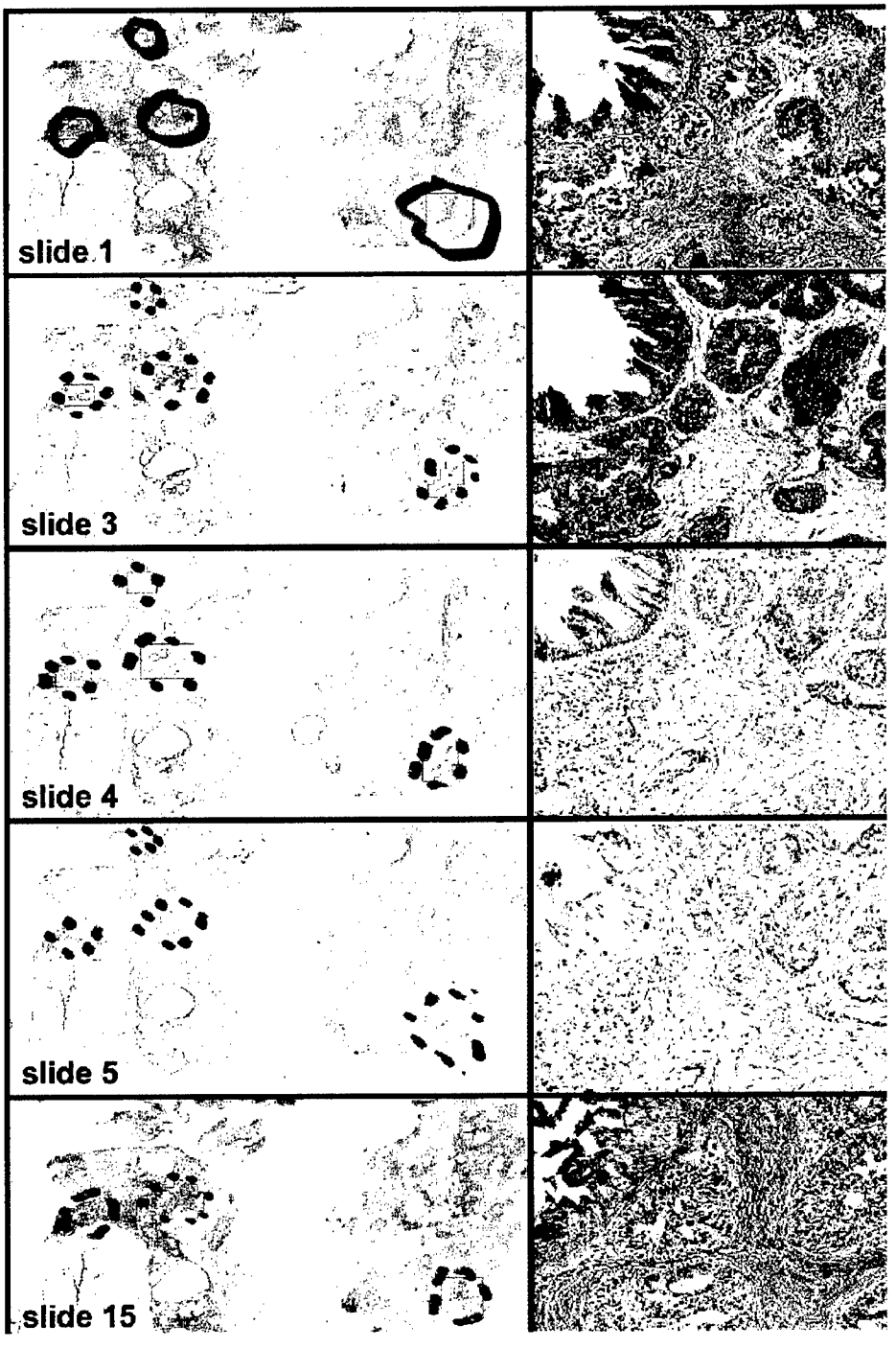



**Figure 1. Immunohistochemistry Analysis.** Fifteen sections were cut from each of the blocks in the Discovery Set and numbered consecutively. Slides 1 and 15 were H&E stained, slide 3 was stained for cyclin D1, slide 4 for Cox-2, and slide 5 dual stained for Ki67 and cleaved caspase 3. Areas of interest on slide 1 were circled by the pathologist, and the corresponding areas were marked on the other slides. Slides were digitized using the BLISS Virtual Microscopy system. Shown on the left are overviews of the slides from one block and on the right are higher magnification images from within the area marked by an asterisk.

## APPENDICES

Our last report contained copies of questionnaires and questionnaire cover letters, a diagram of our relational database, and a copy of our 2003 AACR abstract. We have included in this report a copy of the following:

A. Wayne State IRB minute
B. Our consent form, brochure and questionnaire that is used with fresh tissue collection
C. 2003 AACR abstract
D. 2004 AACR abstract

**Appendix A**

Wayne State IRB Minute

021702M1F Kathryn Carolin Amirika, M.D., Surgery, "C-2479: Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk" Sponsor: Department of Defense/Mayo Clinic. The following changes to the above protocol have been reviewed and approved, effective immediately: The changes were initiated by the PI: Consent Form: (dated 04/22/04) 1) Compensation section has been revised per the DOD. 2) Additional administrative revisions have been made to the consent to bring it more in sync with the Mayo Clinic consent and to clarify the procedures. HIPAA: HIPAA Authorization has been revised to correct the title (dated 03/23/04).

**Principal Investigator:**

Lynn C. Hartmann, M.D.

**Study Coordinator:**

If you have questions please contact:

Mary Amundsen, R.N., M.S.
Toll free number: 1-877-588-9301
8am – 4pm central standard time
e-mail: amundsen.mary@mayo.edu

If no answer, please leave name, number and best time to be called.

**Address:**

Mayo Clinic
Benign Breast Disease Study
Charlton 6
200 First Street SW
Rochester, MN 55905

MAYO CLINIC

200 First Street SW
Rochester, Minnesota 55905
**www.mayoclinic.org**

MC2641-01

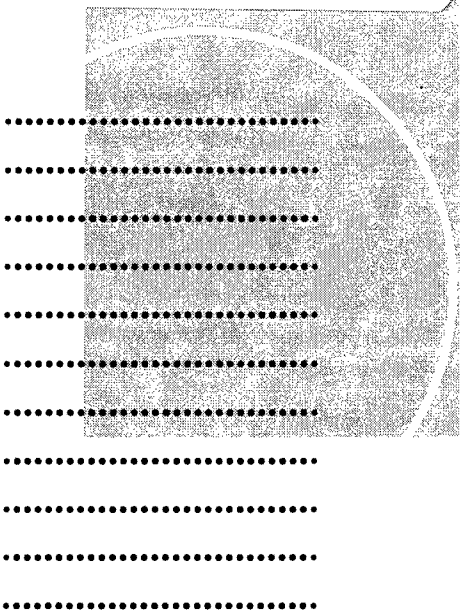


MAYO CLINIC

# *Benign Breast Disease – Tissue Study*

Hartmann, Lynn C., M.D.

Appendix B



## Definition

Benign breast disease is a very common condition and includes all benign conditions found at the time of breast biopsy.

## Description of Study

The purpose of this study is to look at benign changes in breast tissue to identify what is occurring in the cells of the breast tissue. This study will provide information about what stimulates abnormal cell growth in breast tissue.

## Who is Sponsoring this Study

This work has been, and continues to be, sponsored by several groups including the Komen Foundation, the Breast Cancer Research Foundation and the Department of Defense Medical Research branch.

## How to be Involved

At the time of your breast biopsy, excess tissue may be available for research purposes. If all your tissue is needed for diagnostic purposes you will not be included in the study. However, at times, a sample contains more than the pathologist needs and this excess tissue could then be used for this research study. Some of the laboratory studies will be performed by Dr. Thea Tlsty, a breast researcher at the University of California, San Francisco and some at Mayo Clinic, Rochester.

In addition to the study of breast tissue, we would ask you to complete a one-time questionnaire which should only take ten minutes of your time. This questionnaire would provide additional information essential to the analysis of the relationship of benign breast disease and the risk of later development of breast cancer.

## Costs of Participation

There are no monetary costs to you as part of your participation in the study. If you choose not to participate, your present or future medical care and treatment at the Mayo Clinic will not be jeopardized.

## Confidentiality

All records and tissue used in this study will be used only by research personnel and is kept in locked files in a secure area. Identifying information is never sent outside Mayo without written permission. When results of the study are published in a scientific journal participant's identity is not released. This is in keeping with Mayo Clinic's policy of patient confidentiality and monitored by the Institutional Review Board annually.

## Benefit of Study

Your participation in advancing medical science in the area of breast disease and breast cancer has the potential of improving the health of future generations of women.

MAYO CLINIC
Rochester, Minnesota

**Retain in Correspondence**
**Section of Medical Record**

Name and Registration No.

## *Consent Form for Participation in a Research Study*

**TITLE:** "Study of Benign Breast Disease"

**IRB#:** 170-02 00

**RESEARCHER:** Dr. L. C. Hartmann and colleagues

**PROTOCOL LAST APPROVED BY INSTITUTIONAL REVIEW BOARD:** December 16, 2003

**THIS FORM APPROVED:** December 16, 2003

**This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.**

### Why is this research study being done?

This study is being done to study what regulates breast tissue growth, to better understand if there is a possible relationship between benign changes and breast cancer. Laboratory studies will search for changes in genetic material (DNA and chromosomes) and changes in other biologic processes in tissue specimens.

### How many people will take part in this research study?

The plan is to have approximately 160 women take part in this study. Approximately 80 people will take part at Mayo Clinic Rochester and 80 from Wayne State Medical Center in Detroit.

### What will happen in this research study?

At the time of your breast surgery, excess tissue may be available for use for research purposes. For some women, all the tissue is needed for diagnostic purposes (if this is the case with you, then your specimen would not be included in this study). In other women, the resected specimen contains more material than the pathologist needs to make the diagnosis. In this case, we would like to use this excess, already resected, tissue for research purposes. This research involves doing a number of studies in the laboratory,

including studying the growth characteristics of breast tissue. Some of these laboratory studies will be performed by Dr. Thea Tlsty, a breast researcher at the University of California, San Francisco. Specimens from both Mayo Clinic Rochester and Wayne State will be shipped to Dr. Tlsty's laboratory for study. In addition, we ask that you complete a one-time questionnaire designed for this study. This should take approximately 10 minutes of your time to complete.

**How long will I be in this research study?**

Your breast tissue and information that you have provided on the study questionnaire will be used throughout the period of this study, anticipated to last approximately four years. If you reply that you will allow your tissue to be used in future studies (see below), it will be stored in a secure area of the Mayo Clinic. It may be stored indefinitely. If you don't want your tissue to be used in future studies, it will be stored in a secure area for five years after the completion of the study at which time it will be disposed per institutional policy for disposal of tissue. The five-year period allows time for validation of research findings. Questionnaire data will be stored in locked file cabinets in the research area or archival area of the institution for a period of five years. Electronically stored data will be stored in a secured location. Storage of research data for a period of five years is common. This allows the ability to reevaluate and validate findings.

**Are there reasons I might leave this research study early?**

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the researcher if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the researchers or Mayo may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

**Will any biological sample(s) be stored and used for research in the future?**

If there is excess material available, your sample will be kept at Mayo for possible future use. In addition, researchers at Mayo who are not involved with this study may ask to use your sample for future research. You have a say in how your stored sample would be used in future research. You can still take part in the current study without giving your sample for future use. There are exceptions to use of your sample without your permission. These exceptions are: 1) when government rules allow your sample to be used without identifying you, even with a code; and 2) when use of the sample is not considered human subject research. At all other times:

- you can let Mayo use your sample; or
- you can say no to having your sample used by Mayo.

If you agree to allow your sample to be used for further research, the sample may be stored indefinitely. The sample will be stored at Mayo and would be given a code (rather than your name) while it is stored and when it is used in research. This code would allow your sample to be used without anyone knowing that it is your sample just by looking at the label.

There is a very small chance that some commercial value may result from the use of your sample. If that would happen, you will not be offered a share in any profits.

***Please read the following statements and mark your choice:***

1. I permit my sample to be stored and used in future research of breast tissue at Mayo:

☐ Yes ☐ No Please initial here:_____ Date:_____

2. I permit my sample to be stored and used in future research at Mayo to learn about, prevent, or treat any other health problems:

☐ Yes ☐ No Please initial here:_____ Date:_____

If you want your sample destroyed at any time, write to the Administrator of the Mayo Foundation Office for Human Research Protection, 201 Building 4-60, 200 First Street Southwest, Rochester, Minnesota 55905. Mayo has the right to end storage of the sample without telling you. If you move please send your new address to Mayo Clinic Rochester, Section of Registration, 200 First Street Southwest, Rochester, Minnesota 55905.

If you agree to give your sample, it will be the property of Mayo and may be used for research by Dr. Hartmann and other staff at Mayo Clinic. Researchers at other institutions also may one day ask for a part of your sample for other studies.

**How do researchers from other institutions get the sample?**

Researchers from universities, hospitals, and other health organizations conduct research using tissue. They may contact Mayo and request samples for their studies. If you approve release of your sample by checking 'yes' below, Mayo may send the tissue sample(s) and some information about you to researchers who request them, but Mayo will <u>not</u> send your name, address, phone number, social security number, or any other identifying information with the sample. If you allow your sample to be given to researchers at other institutions, it will be given to them with a code number rather than your name. If these researchers use the sample for future research and decide that a test result may be useful for your health care, they may contact the Mayo Clinic and Mayo would then contact you to offer you the choice to learn the test results.

I permit Mayo to give my sample to researchers at other institutions:

***Please mark one box:***
☐ Yes ☐ No Please initial here:_____ Date:_____

**What are the risks of this research study?**

You are being asked to let researchers use breast tissue that has already been removed. There are no recognized risks to allowing the use of this material for research.

**Are there benefits to taking part in this research study?**

This study will not make your health better. The current laboratory studies that will be performed on your specimen(s) are completely investigational at this time. There will be no direct health benefits to you resulting from these studies. The results of this study may improve the medical community's understanding of breast disease such that women in the future may benefit from this work.

**What other choices do I have if I don't take part in this research study?**

This study is only being done to gather information. You may choose not to take part in this study.

**Will I need to pay for the tests and procedures?**

You will not need to pay for any tests and procedures which are done just for this research study. However, you and/or your health plan will need to pay for all other tests and procedures that you would normally have as part of your regular medical care. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this research.

**What happens if I am injured because I took part in this research study?**

There is no risk of physical harm when taking part in this study.

**What are my rights if I take part in this research study?**

Taking part in this research study does not take away any other rights or benefits you might have if you did not take part in the study. Taking part in this study does not give you any special privileges. You will not be penalized in any way if you decide not to take part or if you stop after you start the study. Specifically, you do not have to be in this study to receive or continue to receive medical care from Mayo Clinic.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

**Who can answer my questions?**

You may talk to Dr. Lynn Hartmann at any time about any questions or concerns you have on this study. You may contact Dr. Hartmann (or an associate) by calling the Mayo operator at telephone (507) 284-2511.

You can get more information about Mayo policies, the conduct of this study, or the rights of research participants from Cindy L. Boyer, Administrator of the Mayo Foundation Office for Human Research Protection, telephone (507) 284-2329 or toll free (866) 273-4681.

Authorization To Use And Disclose Protected Health Information

Your privacy is important to us, and we want to protect it as much as possible. By signing this form, you authorize Mayo Clinic Rochester and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol. This information might be in different places, including your original medical record, but we will only disclose information that is related to this research protocol for the purposes listed below.

This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study. If some of the information is reported in published medical journals or scientific discussions, it will be done in a way that does not directly identify you.

The study data sent by the study doctor to the sponsor does not include your name, address, social security number, or other information that directly identifies you. Instead, the study doctor assigns a code number to the study data and may use your initials. Some study data sent to the sponsor may contain information that could be used (perhaps in combination with other information) to identify you (e.g., date of birth). If you have questions about the specific health information that will be sent to the sponsor, you should ask the study doctor.

This information may be given to other researchers in this study (including those at other institutions), or private, state or federal government parties or regulatory authorities (U.S. and other countries) responsible for overseeing this research. These may include the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Foundation Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

If this information is given out to anyone outside of Mayo, the information may no longer be protected by federal privacy regulations and may be given out by the person or entity that receives the information. However, Mayo will take steps to help other parties understand the need to keep this information confidential.

This authorization lasts forever.

You may stop this authorization at any time by writing to the following address:

Mayo Foundation
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

A copy of this form will be placed in your medical record.

**I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this research study.**

| _____ | _____ | _____ |
|---|---|---|
| (Date) | (Printed Name of Participant) | (Clinic Number) |

_____
(Signature of Participant)

| _____ | _____ |
|---|---|
| (Date) | (Printed Name of Individual Obtaining Consent) |

_____
(Signature of Individual Obtaining Consent)

# Breast Biopsy Survey

***Answer every question by darkening the appropriate oval. If you are unsure about how to answer a question, please give the best answer you can.***

**MARKING INSTRUCTIONS:**

- Use a No. 2 pencil only.
- Fill in the number in the box and then darken the oval.
- Darken the oval completely.
- Do not make any stray marks on this form.
- Erase all changes completely.

**CORRECT MARK:** ●


**Please complete today's date:**

| TODAY'S DATE | | |
|---|---|---|
| MONTH | DAY | YEAR |
| ○ Jan | ① ⑪ ㉑ | ○2004 |
| ○ Feb | ② ⑫ ㉒ | ○2005 |
| ○ Mar | ③ ⑬ ㉓ | ○2006 |
| ○ Apr | ④ ⑭ ㉔ | ○2007 |
| ○ May | ⑤ ⑮ ㉕ | ○2008 |
| ○ Jun | ⑥ ⑯ ㉖ | |
| ○ Jul | ⑦ ⑰ ㉗ | |
| ○ Aug | ⑧ ⑱ ㉘ | |
| ○ Sep | ⑨ ⑲ ㉙ | |
| ○ Oct | ⑩ ⑳ ㉚ | |
| ○ Nov | ㉛ | |
| ○ Dec | | |

Place barcode label here.

PLEASE DO NOT WRITE IN THIS AREA

1169

## Medical History

We are interested in your medical history and family history.

1. **At what age did you begin menstruating?**

| Age | |
|---|---|
| 0 | 0 |
| 1 | 1 |
| 2 | 2 |
| 3 | 3 |
| 4 | 4 |
| 5 | 5 |
| 6 | 6 |
| 7 | 7 |
| 8 | 8 |
| 9 | 9 |

2. **Have you had children?**
(Count only live births.)

○ No ○ Yes

**How old were you when your first child was born?**

| Age | |
|---|---|
| 0 | 0 |
| 1 | 1 |
| 2 | 2 |
| 3 | 3 |
| 4 | 4 |
| 5 | 5 |
| 6 | 6 |
| 7 | 7 |
| 8 | 8 |
| 9 | 9 |

**How many children have you had?**

○ 1 ○ 5 ○ 9
○ 2 ○ 6 ○ 10 or more
○ 3 ○ 7
○ 4 ○ 8

**Did you breastfeed any of your children for more than one month?**

○ No ○ Yes

**How many children did you breastfeed for more than one month?**

○ 1 to 2 ○ 6 to 10
○ 3 to 5 ○ 11 or more

3. **Have you had a menstrual period within the last year?**

○ No ○ Yes

**Have you gone through menopause?**

○ No ○ Yes

**At what age did your periods stop?**

| Age | |
|---|---|
| 0 | 0 |
| 1 | 1 |
| 2 | 2 |
| 3 | 3 |
| 4 | 4 |
| 5 | 5 |
| 6 | 6 |
| 7 | 7 |
| 8 | 8 |
| 9 | 9 |

4. **Have your ovaries been surgically removed?**

○ No ○ Yes, one ovary ○ Yes, both ovaries ○ Yes, but unsure if one or both

**At what age was your ovary/ovaries removed?**
(If more than one surgery, please record age at last surgery.)

| Age | |
|---|---|
| 0 | 0 |
| 1 | 1 |
| 2 | 2 |
| 3 | 3 |
| 4 | 4 |
| 5 | 5 |
| 6 | 6 |
| 7 | 7 |
| 8 | 8 |
| 9 | 9 |

**5. Have you ever taken estrogen (hormones) at or after menopause?**

○ No ○ Yes

**If yes, how many total years?**

○ 5 years or less
○ 6 to 10 years
○ 11 years or more

**Are you currently using estrogen (hormones)?**

○ No ○ Yes

**If no, how long ago did you stop?**

○ 5 years ago or less
○ 6 to 10 years ago
○ 11 years ago or more

**6. Have you had a surgical breast biopsy before?** (Do not count needle procedures.)

○ No ○ Yes

**If yes, how many have you had previously?**

**No. of breast biopsies**

**7. How often have you had a mammogram?**

○ Never ○ Regularly ○ Irregularly

**How often?**

○ Every year
○ Every 2 years
○ Every 3 years

**Approximately how many times in the past 10 years?**

**No. of times**

**8. In what year did you have your last (most recent) routine mammogram?**

○ Have never had a mammogram

**Year**

**9. Have you ever had breast reduction surgery?**

○ No ○ Yes

**If yes, at what age was this surgery done?**

**Age**

**10. Have you ever taken tamoxifen or raloxifene (Evista)?** (Mark all that apply.)

○ No ○ Yes, tamoxifen ○ Yes, raloxifene (Evista)

**If yes, what year did you start taking tamoxifen?**

**Year**

**How many total years did you take tamoxifen?**

○ Less than 1 year
○ 1 to 2 years
○ 3 to 4 years
○ 5 years
○ Over 5 years

**If yes, what year did you start taking raloxifene (Evista)?**

**Year**

**How many total years did you take raloxifene (Evista)?**

○ Less than 1 year
○ 1 to 2 years
○ 3 to 4 years
○ 5 years
○ Over 5 years

**11. Have you ever been diagnosed with cancer?**

○ No ○ Yes

**If yes, what type of cancer have you had?**

○ Colon ○ Ovarian ○ Uterine ○ Other, specify: _____

**At what age were you diagnosed?**

Age | Age | Age | Age

**12. Have any of your blood relatives had breast cancer, prostate cancer, colon cancer, or ovarian cancer?**

○ No → **Please skip to question 13.**

○ Yes

**If yes, be sure to mark the space for all blood relatives and each specific cancer. For sisters, daughters, and aunts, please indicate the number who have breast cancer in each group (4+ means 4 or more of these people have breast cancer).**

| Cancer: | Your Sister(s) | Your Brother(s) | Your Daughter(s) | Your Son(s) |
|---|---|---|---|---|
| Colon | ○ | ○ | ○ | ○ |
| Ovarian | ○ | | ○ | |
| Prostate | | ○ | | ○ |
| Breast | ○1 ○2 ○3 ○4+ | ○ | ○1 ○2 ○3 ○4+ | ○ |

Age at diagnosis of breast cancer. (If more than one sister or daughter, age of youngest when diagnosed.) — Age | Age

**Continued next column...**

| Cancer: | Your Mother | Your Mother's Sister(s) | Your Mother's Brother(s) | Your Mother's Mother (Your Grandmother) |
|---|---|---|---|---|
| Colon | ○ | ○ | ○ | ○ |
| Ovarian | ○ | ○ | | ○ |
| Prostate | | | ○ | |
| Breast | ○ | ○1 ○2 ○3 ○4+ | ○ | ○ |

Age at diagnosis of breast cancer. (If more than one aunt, age of youngest when diagnosed.) — Age | Age | Age

| Cancer: | Your Father | Your Father's Sister(s) | Your Father's Brother(s) | Your Father's Mother (Your Grandmother) |
|---|---|---|---|---|
| Colon | ○ | ○ | ○ | ○ |
| Ovarian | | ○ | | ○ |
| Prostate | ○ | | ○ | |
| Breast | ○ | ○1 ○2 ○3 ○4+ | ○ | ○ |

Age at diagnosis of breast cancer. (If more than one aunt, age of youngest when diagnosed.) — Age | Age

| Cancer: | Your Cousin(s) (Mother's Side) | Your Cousin(s) (Father's Side) |
|---|---|---|
| Colon | ○ | ○ |
| Ovarian | ○ | ○ |
| Prostate | ○ | ○ |
| Breast | ○1 ○2 ○3 ○4+ | ○1 ○2 ○3 ○4+ |

Age at diagnosis of breast cancer. (If more than one cousin, age of youngest when diagnosed.) — Age | Age

13. **Have other cancers occurred in blood relatives on your mother's side?**

○ No

○ Yes → **If yes, please list relative and type of cancer.**

_____

_____

_____

14. **Have other cancers occurred in blood relatives on your father's side?**

○ No

○ Yes → **If yes, please list relative and type of cancer.**

_____

_____

_____

15. **What is your current weight?** → **Pounds**

16. **What was your weight at 18 years of age?** → **Pounds**

17. **What is your height?** (Round to the nearest inch.)

**Feet** **Inches**

18. **Have you ever smoked cigarettes on a regular basis, that is, more than 100 cigarettes in your entire life?**

○ No

○ Yes

**If yes, how old were you when you first started smoking cigarettes on a regular basis?**

**Age**

**On average, during the entire time you smoked, how many cigarettes did you smoke per day?**

**Cigarettes per day**

**Do you currently smoke?**

○ No → **At what age did you quit smoking?** **Age**

○ Yes

19. **Have you ever consumed alcohol-containing beverages on a regular basis, that is, at least once per month?** (By alcohol-containing beverages, we mean 1 5-oz. glass of wine, 1 beer, or 1 mixed drink.)

○ No ○ Yes

**If yes, how old were you when you began drinking alcoholic beverages regularly?**

| Age | |
|---|---|
| ⓪ | ⓪ |
| ① | ① |
| ② | ② |
| ③ | ③ |
| ④ | ④ |
| ⑤ | ⑤ |
| ⑥ | ⑥ |
| ⑦ | ⑦ |
| ⑧ | ⑧ |
| ⑨ | ⑨ |

**Do you currently drink alcoholic beverages on a regular basis?**

○ No → **At what age did you quit drinking regularly?**

| Age | |
|---|---|
| ⓪ | ⓪ |
| ① | ① |
| ② | ② |
| ③ | ③ |
| ④ | ④ |
| ⑤ | ⑤ |
| ⑥ | ⑥ |
| ⑦ | ⑦ |
| ⑧ | ⑧ |
| ⑨ | ⑨ |

○ Yes

**What is/was your average consumption of alcoholic beverages?**

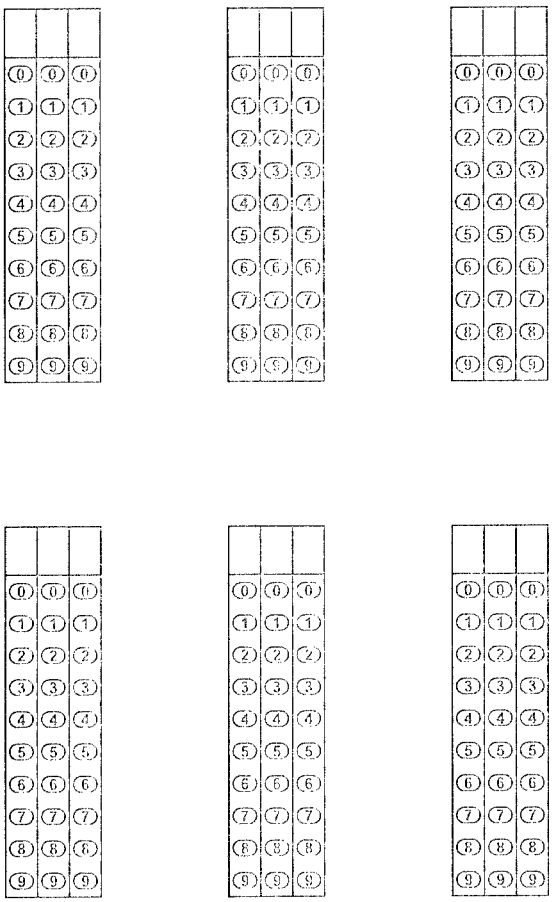
○ Less than one per month
○ 1 to 3 per month
○ 4 to 6 per month
○ 1 per day
○ 2 to 3 per day
○ 4 to 5 per day
○ 6 or more per day

Thank you for taking the time to complete the survey!

PLEASE DO NOT WRITE IN THIS AREA

**FOR OFFICE USE ONLY**

## Family History



PLEASE DO NOT WRITE IN THIS AREA

1169

## Appendix C

**Benign Breast Disease and Breast Cancer Risk**. LC Hartmann[1], D Visscher[1], C Reynolds[1], MH Frost[1], LJ Melton[1], C Vachon[1], T Tlsty[2], D Hillman[1], JL Johnson[1], WL Lingle[1], V Suman[1], TA Sellers[1].

[1] Mayo Clinic Cancer Center, Rochester, MN
[2] University of California, San Francisco, CA

**Introduction**: Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. The ability to identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis would permit more aggressive clinical intervention, including closer surveillance and prevention opportunities. To facilitate this discovery process, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.
**Methods**: The Mayo Clinic Surgical Index was used to identify all women who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/82 and 12/31/91 (n = 5153). The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire was mailed to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.
**Results**: This 10-year cohort includes 5153 women with 66,290 person years of follow-up (through 2/02). The median age at BBD diagnosis was 54 years (13-94), and 41% were age 50 or less. Some family history of breast cancer was present in 32%, while 17% had an affected first-degree relative. Thus far, 255 women are known to have developed BC. The interval from BBD to BC is: ≤ 5 yrs, 33.7%; 5.1-10 yrs, 34.5 %; 10.1-15 yrs, 27.5%; > 15 yrs, 4.3%. The cancer occurred in the same breast as the BBD in 125 women (49%), the opposite breast in 84 (32.9%), and both breasts in 10 (3.9%). Side of BC is pending for 36 (14%) women. The estimated 5-year, 10-year and 15-year breast cancer incidence rates are 1.8% (95%CI: 1.4-2.1%), 3.6% (95%CI: 3.1-4.2%), and 5.8% (95%CI: 5.1-6.5%), respectively. Incorporating time from BBD to cancer and the side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.

**Conclusions**: We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.

## Appendix D

**Benign Breast Disease and Breast Cancer Risk.** LC Hartmann[1], D Visscher[1], MH Frost[1], LJ Melton[1], C Vachon[1], F Couch[1], V Shridhar[1], K Ghosh[1], A Degnim[1], D Hillman[1], V Suman[1], RA Vierkant[1], SD Maloney[1], VS Pankratz[1], T Tlsty[2], TA Sellers[3], WL Lingle[1].

[1]Mayo Clinic Cancer Center, Rochester, MN
[2]University of California, San Francisco, CA
[3]Moffitt Cancer Center, Tampa, FL

**Introduction**: Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. To identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.
**Methods**: The Mayo Clinic Surgical Index was used to identify all women ages 18-85 who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/67 and 12/31/91. The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire is being used to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.
**Results**: This 25-year cohort includes 11,782 women with 181,284 person years of follow-up. The median age at BBD diagnosis was 50.0 years. Some family history of BC was present in 40% of those surveyed; 21% had an affected first-degree relative. Thus far, 705 women are known to have developed BC, at a median of 9.2 years after their BBD. The interval from BBD to BC is <= 5 years, 27%; 5.1 - 10 years, 26%; 10.1-15 years, 24%; >15 years, 23%. The cancer occurred in the same breast as the BBD in 279 women (40%), the opposite breast in 189 (27%) and both breasts in 18 (3%). Side is pending for 219 (31%). The estimated 5-yr, 10-yr and 15-yr BC incidence rates are 2.0%, 4.1%, and 6.4%, respectively for women with BBD from 1982-1991 (follow-up ongoing for 1967-81 group). The histopathologic review has been completed for 3,004 of the BBD specimens. Non-proliferative disease was found in 65.8%, proliferative disease without atypia in 28.6% and atypia (atypial ductal hyperplasia or atypial lobular hyperplasia) in 3%. Incorporating time from BBD to BC, histology, and side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.
**Conclusions**: We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.